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EDITORIAL

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What are the prospects of apolipoprotein profiling for cardiovascular disease?

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1. Lipids and CVD (cardiovascular disease) risk

Lipid abnormalities account for over 60% of the population attributable risk for myocardial infarction and are the most important single target for prevention, along with blood pressure lowering and smoking cessation. The low-density lipoprotein cholesterol (LDL-C) axis is an established therapeutic target in CVD [1]. In contrast to genetic disorders that lead to higher or lower LDL-C, genetic mutations that affected high-density lipoprotein-cholesterol (HDL-C) did not translate into altered CVD risk [2]. Niacin and cholesterol ester transferase protein (CETP) inhibitors (with the recent exception of anacetrapib) that raise HDL-C failed to improve cardiovascular (CV) outcome on top of statins. Research on HDL mainly focused on the antiatherogenic functions of HDL. Although statistically high HDL-C is associated with reduced risk of CVD, some patients with very high HDL-C are actually at increased risk of CVD [3]. Thus, not all HDL elevations are protective.

While there is a well-known inverse correlation between plasma HDL and atherosclerosis since the initial observation by Barr, Russ, and Eder in the early 1950s [4], it is now apparent that HDL-C is not a good surrogate measure for therapeutics or functionality. A consensus is building that HDL function may be a better target than HDL-C. In 1974, Bates and Rothblat demonstrated that HDL can mediate sterol efflux from mouse macrophage cell lines [5]. Cholesterol efflux capacity (CEC) can vary by a factor of two at a given concentration of HDL-C. Thus far, several studies have used CEC [6,7]. In contrast to HDL-C, CEC is inversely correlated to CVD risk and low CEC is an independent risk factor for CVD. This metric of HDL function, however, involves cellular assays, and there is a need for better measurements than CEC that address the diverse functionality of HDL particles. First, the same HDL can result in a range of different read-outs for CEC if incubated with different murine cell types. Second, a cellular assay is less suitable for routine clinical implementation. The assessment of lipoprotein composition is easier to incorporate into routine biomarker assays but must relate to lipoprotein function.

Current laboratory measurements of plasma lipids principally involve total cholesterol, HDL-C and triglycerides. LDL-C is not always measured for CVD risk assessment but calculated according to the Friedewald formula. Among apolipoproteins, the main focus has been on apoB-100 and apoA-1. In comparison, other apolipoproteins have received considerably less attention, with the exception of Lp(a) that has an incremental predictive effect for CVD risk [8,9].

2. Lipidomics applied to epidemiological cohorts

We have recently reported a lipidomic signature of CVD, in which select triglyceride species in whole plasma outperformed cholesterol esters [10]. This was the first prospective, community-based study reporting a systematic analysis of the plasma lipidome in the context of CVD. Using mass spectrometry (MS), we detected 135 different lipid species attributable to 8 different lipid classes in plasma samples of the Bruneck Study. Samples from the UK Twin Registry were used for validation purposes. The study allowed four main conclusions:

(i) There is a broad diversity of potential CV effects of lipid species within most lipid classes, and as a consequence, individual lipids outperform lipid summary measures with regard to CVD risk prediction;
(ii) Lipid species of low carbon number and double-bond content show the strongest and most consistent positive associations with CVD;
(iii) Molecular lipid profiling by MS results in a significant improvement in CVD risk discrimination and classification beyond the information provided by classic risk factors, including conventional lipid measures; and
(iv) The stronger association of certain lipid species with CVD can, at least in part, be explained by a shift in the plasma fatty acid composition.

The importance of individual lipid species in the context of CVD may have been underestimated by an unwarranted focus on lipid classes. Based on the traditional lipid measures of total triglycerides, total cholesterol, LDL-C, and HDL-C, our understanding of the role of lipids in the pathophysiology of CVD is mainly confined to lipid classes rather than detailed molecular entities (i.e. the type of fatty acids that are conjugated to the glycerol backbone). Thus, different molecular lipid species exert different biological effects, and important information may be missed by sum measurements such as total triglycerides. In contrast to cholesterol, triglycerides were long thought to be merely a risk marker for CVD, in part due to their inverse

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correlation with HDL-C. Evidence from two recent Mendelian randomization studies [11,12] support a primary role of triglyceride-rich lipoproteins (TRL) for CVD. Loss-of-function mutations in apoC-3 reduce triglyceride levels [13] and result in a 40% reduction of CVD [11,12]. It is thought that TRL remnants can penetrate the arterial intima, and that their cholesterol content is potentially more atherogenic than that of LDL [14]. Importantly, TRL lipoproteins carry apoB-100. Current measurements for apoB do not discriminate between LDL and other apoB-100 carrying lipoprotein particles, including TRL. Thus, there is an unmet need for more comprehensive measurements that address the diverse functionality of apolipoprotein particles. Lipoprotein composition, which might be easier to develop into a routine biomarker, must relate to lipoprotein function.

3. Proteomics applied to epidemiological cohorts

In addition to lipidomic profiling, we have also used a multiple reaction monitoring–MS (MRM-MS) platform for apolipoprotein profiling. MRM-MS was selected by Nature Methods as technology of the year in 2012. MRM-MS allows measurements of an unprecedented number of apolipoproteins with high specificity and without the constraints of antibodies. Due to its selectivity, the technology is the 'gold standard' in the quantitation of small molecules. MRM-MS is already used in clinical applications such as screens for inborn errors of metabolism, drug abuse, toxicology, and monitoring plasma concentrations of therapeutic drugs. More recently, its amenability for high-throughput analysis of plasma proteins in epidemiological research has become apparent. Using MRM-MS, we simultaneously measured 13 apolipoproteins (apoA-1, apoA-2, apoB-100, apoA-4, apoC-1, apoC-2, apoC-3, apoD, apoE, apoH, apoJ, apoL-1, and apoM) in the Bruneck study, an ongoing prospective community-based survey that ranks among the most successful epidemiological studies on CVD [15]. The Bruneck Study offers 25 years of follow-up with detailed clinical assessments and samples collected every 5 years (age range of participants at baseline: 40–79 years, N = 1,000). Lipid variables were clustered using agglomerative hierarchical clustering on the basis of complete linkage, defining the distance between 2 variables as 1 minus their correlation. Within the dependency structure of plasma apolipoproteins, multiple nested clusters emerged. Clusters are groups of variables with high intercorrelations and similar correlations with the other variables. One of the most prominent clusters included apoB-100, non-HDL cholesterol, apoE, triglycerides, apoC-2, and apoC-3, thus reflecting apoB-containing lipoproteins. This cluster contained two sub clusters with apoC-2, apoC-3, apoE, triglycerides (representing VLDL) and apoB-100, non-HDL-C (representing LDL). Other notable clusters included apoA-2, apoM, and apoC-1, HDL cholesterol and apoA-1. After adjustment for age, sex, and statin therapy, the strongest association with incident CVD was not observed for apoB-100, but for three other apolipoproteins: apoC-3 (hazard ratio per 1 standard deviation higher level, 95% confidence interval: 1.38, 1.17 to 1.63), apoC-2 (1.40, 1.17 to 1.67), and apoE (1.31, 1.13 to 1.52; all $P < 0.001$). The associations for apoC-3, apoC-2, and apoE were attenuated but remained significant under adjustment for CVD risk factors, including the conventional lipid measures such as HDL and non-HDL cholesterol as well as systolic blood pressure, type 2 diabetes and smoking. Our finding that not apoB-100 but C apolipoproteins and apoE had the most significant associations with CVD underscores the importance of TRLs for the pathogenesis of CVD. Similar results have since been reported from a larger prospective cohort with 832 coronary artery disease cases and 1879 controls: when investigating apoC-3 levels using antibody-based methods in 2711 participants, Capelleveen and colleagues found apoC-3 levels to be higher in cases compared with controls. The association of apoC-3 levels with incident CAD risk was independent of traditional CVD risk factors. ApoC-3 was strongly associated with higher VLDL as well as intermediate-density lipoprotein particle numbers, larger VLDL mean particle size, a shift toward small dense LDL particle size, and inflammation[16].

4. Future outlook

Currently, apolipoproteins are not directly measured in patients with CVD. The function and metabolism of lipoproteins is governed by their apolipoproteins, yet there was no attempt to quantify a comprehensive panel of plasma apolipoproteins by MRM-MS and assess their comparative association with future CVD in the general community. Previous studies have related plasma levels of individual apolipoproteins to CVD with the use of available immunoassays. Historically, the focus was mostly restricted to apoB-100 and apoA-1, even though other apolipoproteins, in particular apoC-3, have been independently linked to CVD. We capitalized on our proteomics expertise by applying MRM-MS to measure apolipoproteins in a community-based study. Among a broad panel of plasma apolipoproteins quantified by MRM-MS, VLDL-associated apolipoproteins were most significantly related to CVD. There is an unmet need for comprehensive apolipoprotein and lipidomics measurements. With the advent of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors and novel RNA therapeutics, there is a renewed interest in lipid metabolism and CVD. For example, we determined the effects of apoC-3 inhibition by Volanesorsen, a second-generation antisense drug targeting apoC-3, using samples from 2 human intervention trials (IONIS1 and IONIS2) [15]. As expected, MRM-MS showed reduced plasma levels of apoC-3 but also revealed reductions in apoC-2 and apoE. Along with lower triacylglycerols, lower diacylglycerol levels were observed. Thus, more comprehensive apolipoprotein measurements are needed not only in epidemiological cohorts to obtain a better understanding of the associations of apolipoproteins with CVD risk, but also in clinical trials for improved patient selection for evaluating treatment efficacy and safety.

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Declaration of interest

M. Mayr is a named inventor on patents detailing claims to molecular lipid species and cardiovascular risk. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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